```
? d s
Set     Items   Description
S1          8   S HBV AND DRUG AND (RESISTANCE OR RESISTANT) AND YSDD
S2          5   RD  (unique items)
S3          5   S HBV AND DRUG AND (RESISTANCE OR RESISTANT) AND YSDD
AND SERINE
S4          2   S S3 NOT S2
S5          1   RD  (unique items)
S6         14   S HBV AND YSDD
S7          6   RD  (unique items)
S8          2   S S7 NOT S2
S9         49   S HBV AND L180M
S10        19   RD  (unique items)
```

10/3,AB/1 (Item 1 from file: 155) **Links**
Fulltext available through: USPTO Full Text Retrieval Options SCIENCEDIRECT
MEDLINE(R)
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22501000 **PMID:** 17074014

YMDD motif variants in inactive hepatitis B carriers detected by Inno-Lipa HBV DR assay.

Akarsu Mesut; Sengonul Aylin; Tankurt Ethem; Sayiner Ayca Arzu; Topalak Omer; Akpinar Hale; Abacioglu Yusuf Hakan
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Journal of gastroenterology and hepatology (Australia) Dec 2006 , 21 (12) p1783-8 ,
ISSN: 0815-9319--Print **Journal Code:** 8607909
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
INTRODUCTION: Mutations of hepatitis B virus (**HBV**) polymerase, especially occurring at the highly conserved YMDD region, are related to resistance to lamivudine. Although these mutations are frequently secondary to lamivudine use, they can also occur naturally. The aim of the present study was to determine the prevalence of YMDD variants that exist naturally in patients who are inactive **HBV** carriers. METHODS: Seventy-one adult inactive **HBV** carriers were studied. All of the patients were confirmed to have maintained normal alanine aminotransferase (ALT) values for one or more years by monitoring serum ALT levels at 3-monthly intervals. None of the patients received interferon or antiviral agents. YMDD variants were analyzed by the **HBV** Drug Resistance Line Probe assay (Inno-Lipa **HBV**-DR). RESULTS: YMDD variants were detected in 13 (18.3%) of the 71 anti-HBe positive inactive **HBV** carriers. Of the 13 patients, 10 (76.9%) also had accompanying **L180M** mutation. The combination of wild type and YMDD variant **HBV** was present in 11 of 13 patients. In two patients, only YIDD and/or YVDD variants plus **L180M** were detected without the presence of wild YMDD motif. CONCLUSION: Naturally occurring YMDD motif variants were

detected at a high rate in a group of lamivudine-untreated inactive **HBV** carriers.

10/3,AB/2 (Item 2 from file: 155) **Links**
Fulltext available through: USPTO Full Text Retrieval Options SCIENCEDIRECT
MEDLINE(R)
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22336301 **PMID:** 17030194
Selection of a multiple drug-resistant hepatitis B virus strain in a liver-transplanted patient.

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Gastroenterology (United States) Oct 2006 , 131 (4) p1253-61 , **ISSN:** 0016-5085--Print
Journal Code: 0374630
Publishing Model Print-Electronic
Document type: Case Reports; Journal Article; Research Support, Non-U.S. Gov't
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
BACKGROUND & AIMS: Sequential anti-hepatitis B virus (**HBV**) therapy may lead to the selection of complex mutants. We analyzed the genetic and phenotypic evolution of the viral quasispecies of a patient who received successively lamivudine, add-on adefovir+lamivudine, followed by lamivudine+adefovir+hepatitis B immunoglobulins (HBIg) after orthotopic liver transplantation. METHODS: For genotypic analysis, a 1310-bp region of the polymerase gene was amplified, cloned, and sequenced. Huh-7 cells were transfected to compare the replication fitness of **HBV** mutants and their susceptibility to drugs. RESULTS: At baseline, all **HBV** genomes carried a wild-type (wt) RT gene but 22% harbored the sP120S and 55% the sC107stop mutations within the surface (S) gene associated with vaccine escape. Following viral breakthrough to lamivudine monotherapy, a complex mixture of lamivudine-resistant **HBV** strains prevailed. Interestingly, among these mutants emerged a population harboring only the rtL180M+A181V mutations, conferring lamivudine-resistance in vitro. After addition of adefovir to the ongoing treatment, viral load dropped, and the patient underwent an orthotopic liver transplantation and received HBIg. As viral load rose again, a single viral population was progressively selected, harboring the rtV173L+**L180M**+A181V+N236T and sP120S mutations. In vitro, this last mutant showed a level of replication reduced by only 30% compared to wt **HBV** and a strong resistance to both lamivudine (>1000-fold) and adefovir (>10-fold). It remained sensitive to tenofovir both in vitro and in vivo. CONCLUSIONS: We report the selection of a complex **HBV** mutant that escaped the antiviral pressure of lamivudine, adefovir, and HBIg, and provide insight on the process of selection via genotypic and phenotypic analysis.

10/3,AB/3 (Item 3 from file: 155) **Links**
Fulltext available through: American Chemical Society USPTO Full Text Retrieval Options SCIENCEDIRECT
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20571262 **PMID:** 16539393

Inhibition of hepatitis B virus (HBV) replication by pyrimidines bearing an acyclic moiety: effect on wild-type and mutant HBV.

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Journal of medicinal chemistry (United States) Mar 23 2006 , 49 (6) p2049-54 , **ISSN:** 0022-2623--Print **Journal Code:** 9716531
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Chronic hepatitis B virus (**HBV**) infection remains a major health problem worldwide. The main clinical limitation of a current antiviral drug for **HBV**, lamivudine, is the emergence of drug-resistant viral strains upon prolonged therapy. A group of 5-, 6-, or 5,6-substituted acyclic pyrimidine nucleosides with a 1-[(2-hydroxyethoxy)methyl] moiety were synthesized and evaluated for antiviral activities. The target compounds were prepared by the reaction of silylated uracils possessing a variety of substituents at the C-5 or C-6 positions or both with 1,3-dioxolane in the presence of potassium iodide and chlorotrimethylsilane by a convenient and single-step synthesis. Among the compounds tested, 5-chloro and 5-bromo analogues possessing an acyclic glycosyl moiety were the most effective and selective antiviral agents in the in vitro assays against wild-type duck **HBV** (EC50=0.4-2.2 and 3.7-18.5 microM, respectively) and human **HBV**-containing 2.2.15 cells (EC50=4.5-45.4 and 18.5-37.7 microM, respectively). These compounds were also found to retain sensitivity against lamivudine-resistant **HBV** containing a single mutation (M204I) and double mutations (**L180M**/M204V). The compounds investigated did not show cytotoxicity to host HepG2 and Vero cells, up to the highest concentration tested. The results presented here confirm and accentuate the potential of acyclic pyrimidine nucleosides as anti-**HBV** agents and extend our previous observations. We herein report the capability of acyclic pyrimidine nucleosides to inhibit the replication of both wild-type and drug-resistant mutant **HBV**.

10/3,AB/4 (Item 4 from file: 155) **Links**
Fulltext available through: Oxford University Press USPTO Full Text Retrieval Options SCIENCEDIRECT
MEDLINE(R)
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20146139 **PMID:** 16373428

Genotypic resistance to lamivudine among hepatitis B virus isolates in Mexico.

Alvarado-Esquivel Cosme; de la Ascension Carrera-Gracia Manuela; Conde-Gonzalez Carlos Jesus; Juarez-Figueroa Luis; Ruiz-Maya Lilia; Aguilar-Benavides Sergio; Torres-Valenzuela Alejandro; Sablon Erwin
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Journal of antimicrobial chemotherapy (England) Feb 2006 , 57 (2) p221-3 , **ISSN:** 0305-7453--Print **Journal Code:** 7513617

Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
BACKGROUND: Drug resistance of hepatitis B virus (**HBV**) is an increasing clinical problem. Resistance to lamivudine in **HBV** isolates in Mexico has been poorly explored. OBJECTIVES: To characterize the mutation patterns associated with genotypic resistance to lamivudine and their prevalence among **HBV** isolates in Mexico. MATERIAL AND METHODS: Thirty-nine Mexican **HBV** isolates were analysed by PCR and line probe assay for detection of genetic variants in the polymerase open reading frame domains B and C (INNO-LiPA **HBV** DR; INNOGENETICS N. V., Ghent, Belgium). This assay detects wild-type and mutations at codons 180, 204 and 207 of the **HBV** polymerase gene, and at codon positions 171, 172, 195, 196, 198 and 199 of the **HBV** surface antigen (HBsAg). **HBV** isolates were obtained from HBsAg-positive serum samples of 15 chronic hepatitis patients, two haemodialysis patients with chronic **HBV** carriage, 20 men found positive for HBsAg when seeking HIV testing and two AIDS patients with chronic **HBV** infection. None of the participants had received antiviral therapy. RESULTS: Overall, **HBV** wild-type was found in 37 (94.9%) out of the 39 isolates studied. Two (5.1%) out of the 39 isolates showed mixed wild-type and mutant populations. These mutations occurred in isolates from one hepatitis patient and one haemodialysis patient. The isolate from the hepatitis patient showed a double mutation at codon positions 180 (**L180M**) and 204 (M204V), thus a 2.6% prevalence of genotypic resistance to lamivudine was found. The isolate from the haemodialysis patient showed a single mutation at codon position 180 (**L180M**). The two **HBV** mutant isolates were further analysed for genotype and both isolates were genotype H. CONCLUSIONS: **HBV** genotypic resistance to lamivudine exists in Mexican isolates. The results highlight the importance of testing for **HBV** resistance before treatment and have implications for a more rational use of drugs.

10/3,AB/5 (Item 5 from file: 155) **Links**
Fulltext available through: custom link USPTO Full Text Retrieval Options SCIENCEDIRECT
MEDLINE(R)
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19708127 **PMID:** 16314804
Heart transplantation in patients with chronic hepatitis B: clinical evolution, molecular analysis, and effect of treatment.

Zampino Rosa; Marrone Aldo; Ragone Enrico; Costagliola Loredana; Cirillo Grazia; Karayiannis Peter; Ruggiero Giuseppe; Utili Riccardo
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Transplantation (United States) Nov 15 2005 , 80 (9) p1340-3 , **ISSN:** 0041-1337--Print
Journal Code: 0132144
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: MEDLINE; Completed
We evaluated clinical evolution and hepatitis B virus (**HBV**) molecular changes in heart recipients with chronic **HBV** infection before transplantation, and studied the effects of lamivudine treatment in patients who experienced **HBV** reactivation. Nine patients with chronic **HBV** infection who underwent heart transplantation were investigated. **HBV** surface/core-promoter/precore/core regions were sequenced. Prior to transplantation, all nine patients had consistently normal ALT and low **HBV**-DNA levels. Seven experienced **HBV** reactivation after transplantation (ALT elevated, **HBV**-DNA>200.000 cps/ml). Lamivudine treatment was initially effective in all patients; three patients during the second year of treatment developed lamivudine resistance-associated mutations (rt-**L180M**, rt-M204V) with severe disease reactivation, remitted after switch to adefovir treatment. No other significant **HBV** mutations were identified in the genomic regions studied. Immune suppression is crucial in the reactivation of previous inactive **HBV** infection and in the liver disease progression in heart recipients. Preemptive lamivudine treatment could be useful in the early management of these patients.

10/3,AB/6 (Item 6 from file: 155) **Links**
Fulltext available through: John Wiley and Sons USPTO Full Text Retrieval Options SCIENCEDIRECT
MEDLINE(R)
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15480184 **PMID:** 15915463
Susceptibility to antivirals of a human HBV strain with mutations conferring resistance to both lamivudine and adefovir.

Brunelle Marie-Noelle; Jacquard Anne-Carole; Pichoud Christian; Durantel David; Carrouee-Durantel Sandra; Villeneuve Jean-Pierre; Trepo Christian; Zoulim Fabien
INSERM U271, Laboratoire des virus hepatiques et pathologies associees, Lyon, France.
Hepatology (Baltimore, Md.) (United States) Jun 2005 , 41 (6) p1391-8 , **ISSN:** 0270-9139--Print **Journal Code:** 8302946
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Mutations within the hepatitis B virus (**HBV**) polymerase gene conferring drug-resistance are selected during prolonged lamivudine (3TC) or adefovir dipivoxil (ADV) treatment. Because there is no other approved drug against **HBV**, treatments with 3TC or ADV are used either sequentially or in addition, depending on treatment response or failure. Considering the use of de novo or add-on 3TC+ADV bitherapy, we investigated the possibility of the emergence of an **HBV** strain harboring polymerase mutations conferring resistance to both 3TC (rtL180M+M204V) and ADV (rtN236T). We constructed the **L180M**+M204V+N236T mutant and determined its replication capacity and its susceptibility to different nucleos(t)ide analogs in transiently transfected hepatoma cell lines. The triple mutant replicates its genome in vitro, but less efficiently than either the wild-type (wt) **HBV** or **L180M**+M204V and N236T mutants. Phenotypic assays indicated that the **L180M**+M204V+N236T mutant is resistant to pyrimidine analogs (3TC, -FTC, beta-L-FD4C, L-FMAU). Compared with wt **HBV**, this mutant displays a 6-fold decreased susceptibility to ADV and entecavir and a 4-

fold decreased susceptibility to tenofovir. Interferon alfa inhibited equally the replication of wt and **L180M**+M204V+N236T **HBV**. In conclusion, the combination of rtL180M+M204V and rtN236T mutations impairs **HBV** replication and confers resistance to both 3TC and ADV in vitro. These results suggest that the emergence of the triple mutant may be delayed and associated with viral resistance in patients treated with 3TC+ADV. However, other nucleos(t)ide analogs in development showed an antiviral activity against this multiresistant strain in vitro. This provides a rationale for the clinical evaluation of de novo combination therapies.

10/3,AB/7 (Item 7 from file: 155) **Links**
Fulltext available through: ScienceDirect (Elsevier) USPTO Full Text Retrieval Options SCIENCEDIRECT
MEDLINE(R)
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15303924 **PMID:** 15664258
Sustained HBs seroconversion during lamivudine and adefovir dipivoxil combination therapy for lamivudine failure.

Maynard Marianne; Parvaz Parviz; Durantel Sandra; Chevallier Michele; Chevallier Philippe; Lot Muriel; Trepo Christian; Zoulim Fabien
Liver Department, Hotel-Dieu, Lyon, France.
Journal of hepatology (England) Feb 2005 , 42 (2) p279-81 , **ISSN:** 0168-8278--Print
Journal Code: 8503886
Publishing Model Print
Document type: Case Reports; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
We describe the case of a patient with chronic hepatitis B who became resistant to lamivudine and was treated successfully with adefovir dipivoxil in addition to lamivudine. Lamivudine resistance was associated with the selection of a **L180M**+M204V polymerase mutant. After the addition of adefovir dipivoxil, serum **HBV** DNA levels dropped by more than 4log(10), which was followed by HBsAg clearance after 22 months of combination therapy. Moreover, anti-HBs antibody titers rose above 1000 mIU/mL after 32 months of the new treatment regimen. In parallel, **HBV** DNA declined below 100 copies/mL by a quantitative real time PCR assay. Analysis of intrahepatic viral DNA showed a significant decline of total **HBV** DNA and cccDNA which was accompanied by a decrease of the number of infected cells expressing viral antigens below the detection limit of immunostaining. In parallel, liver histology analysis showed an improvement in both the activity index and fibrosis score. This report suggests that in patients who previously failed lamivudine therapy, proactive antiviral treatment may lead to a beneficial virological and clinical effect.

10/3,AB/8 (Item 8 from file: 155) **Links**
Fulltext available through: USPTO Full Text Retrieval Options SCIENCEDIRECT
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15173059 **PMID:** 15544736

[Determination of hepatitis B virus genotype and detection of lamivudine-resistance mutations]

Determinacion del genotipo del virus de la hepatitis B y deteccion de mutaciones de resistencia al tratamiento con lamivudina.

Nogales M C; Serrano M C; Suarez E; Corpas R; Perez L; Claro R; Jarana R; Romero-Gomez M; Martin-Mazuelos E
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Gastroenterologia y hepatologia (Spain) Nov 2004 , 27 (9) p515-20 , **ISSN:** 0210-5705--Print **Journal Code:** 8406671
Publishing Model Print
Document type: Journal Article ; English Abstract
Languages: SPANISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed

OBJECTIVES: To determine hepatitis B virus (**HBV**) genotypes in southern Seville (Spain) and investigate the development of lamivudine-resistance mutations by using a hybridization technique with specific probes and by comparing the results with those of the direct sequencing technique. To evaluate the temporal relationship between variations in the level of **HBV**-DNA and detection of mutant variants. To analyze the influence of several genotypes on the pattern of mutations developed and on values of viral load and alanine aminotransferase (ALT) after their development. PATIENTS AND METHOD: In 37 patients with chronic **HBV** infection, **HBV** genotype was determined using the LiPA technique. In 10 of these patients undergoing lamivudine treatment for a mean of 19.2 months, the development of lamivudine-resistant mutations was investigated. In these 10 patients, the LiPA technique was compared with direct sequencing. During lamivudine treatment, we determined **HBV** -DNA by polymerase chain reaction (PCR) and ALT every 3-6 months. RESULTS: The most frequent genotypes were D (45.9%) and A (18.9%); 2 patients were genotype B while 18.9% had mixed genotypes. Sequencing showed identical results except in one mixed genotype. Mutations were found in 60% of the cases. The results of sequencing were in agreement, except in the detection of mixed populations composed of mutants and wild-type (WT). Patients with genotype A showed the pattern M204I+WT in the first 12 months and those with genotype D showed the pattern **L180M**+M204V with or without WT at 18 months. In 5/6 cases, an increase of > 1 log10 in **HBV**-DNA was observed 3-8 months before the mutation was detected by LiPA. In patients with genotype B, levels of **HBV**-DNA and ALT after the development of mutations was lower than basal levels and was also lower than those in patients with genotypes A and D. CONCLUSIONS: The LiPA technique for determination of **HBV** genotype and detection of lamivudine-resistance mutations shows excellent correlation with the most complex sequencing technique. Genotype D predominates in southern Seville. During lamivudine treatment, an increase in the level of **HBV**-DNA detected by PCR predicts the development of mutations before these are demonstrated by LiPA.

10/3,AB/9 (Item 9 from file: 155) **Links**
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MEDLINE(R)

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15051410 **PMID:** 15338371

Prognostic indicators of breakthrough hepatitis during lamivudine monotherapy for chronic hepatitis B virus infection.

Tanaka Yasuhito; Yeo Anthony E T; Orito Etsuro; Ito Kiyoaki; Hirashima Noboru; Ide Tatsuya; Sata Michio; Mizokami Masashi
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Journal of gastroenterology (Japan) Aug 2004 , 39 (8) p769-75 , **ISSN:** 0944-1174--Print
Journal Code: 9430794
Publishing Model Print; Comment in J Gastroenterol. 2004 Aug;39(8) 813-4; Comment in PMID 15338383
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
BACKGROUND: Breakthrough hepatitis (BTH), defined as a flare of transaminases alanine aminotransferase [ALT]) can occur during lamivudine monotherapy for hepatitis B virus (**HBV**) infection. There have been many reports of lamivudine-resistant mutations within the C domain of the viral reverse transcriptase; however, the appearance of these mutants is not necessarily correlated with BTH during lamivudine therapy. METHODS AND RESULTS: Entire serial **HBV** genomic sequences before and during lamivudine therapy for 4 patients with BTH and 1 patient without BTH were analyzed and showed changes in the pre-S region. These changes may be associated with ALT flares. Further investigation in a cohort of 36 patients with a median treatment period of 25 months showed that 21 patients had a rise in **HBV**-DNA titer, of whom 18 had BTH. Univariate statistical analyses showed that possible prognostic indicators for the occurrence of BTH were pre-S deletions (P = 0.03) and **L180M**/M204L mutations (P = 0.04). By multivariate Cox regression analyses, significant variables were pre-S deletions (hazard ratio, 0.17; 95% confidence internal (CI), 0.044-0.66) and precore mutations (hazard ratio, 5.70; 95% CI, 1.74-18.71) prior to the commencement of lamivudine monotherapy. Interestingly, BTH occurred after the selection of the wild-type species in the pre-S region during lamivudine monotherapy. CONCLUSIONS: These results suggest that patients with **HBV** pre-S deletion mutants should be monitored carefully during lamivudine therapy.

10/3,AB/10 (Item 10 from file: 155) **Links**
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15012247 **PMID:** 15288618

A novel pattern (sW195a) in surface gene of HBV DNA due to YSDD (L180M plus M204S) mutation selected during lamivudine therapy and successful treatment with adefovir dipivoxil.

Bozdayi A Mithat; Eyigun Can Polat; Turkyilmaz Ahmet R; Avci Ismail Yasar; Pahsa Alaaddin; Yurdaydin Cihan

Journal of clinical virology - the official publication of the Pan American Society for Clinical Virology (Netherlands) Sep 2004 , 31 (1) p76-7 , **ISSN:** 1386-6532--Print **Journal Code:** 9815671
Publishing Model Print
Document type: Letter
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed

10/3,AB/11 (Item 11 from file: 155) **Links**
Fulltext available through: USPTO Full Text Retrieval Options SCIENCEDIRECT
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14987865 **PMID:** 15259898

In vitro susceptibility of lamivudine-resistant hepatitis B virus to adefovir and tenofovir.

Lada Olivier; Benhamou Yves; Cahour Annie; Katlama Christine; Poynard Thierry; Thibault Vincent
Virology, AP-HP Pitie-Salpetriere Hospital, Paris, France.
Antiviral therapy (England) Jun 2004 , 9 (3) p353-63 , **ISSN:** 1359-6535--Print
Journal Code: 9815705
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Emergence of lamivudine-resistant hepatitis B virus (**HBV**) is a major concern in human immunodeficiency virus (HIV) and **HBV** coinfected patients. Following selection of resistant mutants, hepatitis flare or rapid progression to cirrhosis may occur. Treatment of patients with new nucleotide analogues such as adefovir dipivoxil (ADV) or tenofovir disoproxil fumarate (TDF) has shown good efficacy in controlling wild-type or lamivudine-resistant **HBV** replication. The purpose of this study was to assess the in vitro efficacy of new nucleotide analogues on **HBV** strains isolated from lamivudine-treated patients. After purification of **HBV** DNA from patient sera, the whole **HBV** genome was PCR-amplified and cloned. Drug sensitivity was measured after transfection of the isolated full genomes into HepG2 cells and measurement of HBeAg, HBsAg and viral replication in the culture media under increasing drug concentrations. A wild-type strain isolated from an untreated patient served as control. In a clinical study of ADV (Gilead 460i study), seven of the 35 patients carried **HBV** strains with the triple lamivudine resistance-associated amino-acid changes rtV173L/**L180M**/M204V at baseline. Although all patients responded to ADV in this clinical study, the serum **HBV** reduction was lower in the seven patients with the triple mutation (median -3.3 log copies/ml) compared to the patients who had only the rtL180M/M204V mutations (median -4.1 log copies/ml) at week 48 (P=0.04, Mann-Whitney test). In our in vitro system, lamivudine IC50 on lamivudine-resistant **HBV** carrying amino-acid substitutions rtL180M and rtM204V within the polymerase encoding region increased by more than 16,000-fold (from 6 nM to over 100 microM) when compared to wild-type **HBV**. For ADV and TDF, comparison of wild-type and lamivudine-resistant **HBV** IC50 (rtL180M-

M204V) showed, respectively, 2.85-fold (from 0.07 to 0.2 microM) and 3.3-fold (from 0.06 to 0.2 microM) increases, indicating a mild decrease of both drug activities, in vitro. At the ADV concentration of 0.1 microM, presence of the V173L mutation reduced the inhibition of HBsAg production from 50 to 30% (P<0.01) and the viral replication from 45 to 32% (P<0.01, Mann-Whitney). Conversely, tenofovir had similar potency on both **HBV** mutation profiles with 60% inhibition of HBsAg production and 45% inhibition of viral replication at 0.1 microM. Our study supports the high efficacy of ADV and TDF seen in patients after lamivudine breakthrough. The excellent activity of TDF on lamivudine-resistant virus independently of the resistance mutation profile offers an interesting treatment alternative to HIV-**HBV** coinfected patients.

10/3,AB/12 (Item 12 from file: 155) **Links**
Fulltext available through: ScienceDirect (Elsevier) USPTO Full Text Retrieval Options SCIENCEDIRECT
MEDLINE(R)
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14609350 **PMID:** 14642631

Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient.

Villeneuve Jean-Pierre; Durantel David; Durantel Sandra; Westland Christopher; Xiong Shelly; Brosgart Carol L; Gibbs Craig S; Parvaz Parviz; Werle Bettina; Trepo Christian; Zoulim Fabien
Division of Hepatology, Hopital Saint-Luc du Centre Hospitalier Universitaire de Montreal, Montreal, Quebec, Canada
Journal of hepatology (England) Dec 2003 , 39 (6) p1085-9 , **ISSN:** 0168-8278--Print
Journal Code: 8503886
Publishing Model Print
Document type: Case Reports; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
BACKGROUND/AIMS: In contrast to lamivudine, adefovir dipivoxil (ADV) therapy is associated with delayed and infrequent selection of drug resistant hepatitis B virus (**HBV**). METHODS: A 52 year-old man was treated with lamivudine for an **HBV** recurrence on his liver graft. A viral breakthrough was observed and the patient received ADV. Serum **HBV** DNA decreased rapidly and lamivudine was discontinued while ADV monotherapy was maintained. Serum **HBV** DNA levels remained suppressed until a second breakthrough was observed. Lamivudine was then reintroduced together with ADV, and serum **HBV** DNA became undetectable by polymerase chain reaction. RESULTS: Sequence analyses of the **HBV** polymerase gene revealed a sequential selection of lamivudine resistance mutations **L180M**+M204V, followed by a reversion to wild-type, and subsequently the selection of a novel adefovir resistance mutation N236T. Phenotypic analyses in cell culture assays demonstrated that the **HBV** isolates at the time of ADV breakthrough had reduced susceptibility to ADV. This mutant remained sensitive to lamivudine, entecavir and emtricitabine in vitro. CONCLUSIONS: We describe the first case of sequential selection of lamivudine and adefovir resistant strains of **HBV** in a liver transplantation patient. The selection of the N236T polymerase mutant was associated with resistance to ADV but

remained sensitive to lamivudine in vitro and in vivo.

10/3,AB/13 (Item 13 from file: 155) **Links**
Fulltext available through: American Society for Microbiology USPTO Full Text Retrieval Options SCIENCEDIRECT
MEDLINE(R)
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14315342 **PMID:** 12760870
Generation of stable cell lines expressing Lamivudine-resistant hepatitis B virus for antiviral-compound screening.

Walters Kathie-Anne; Tipples Graham A; Allen Marchelle I; Condreay Lynn D; Addison William R; Tyrrell Lorne
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Antimicrobial agents and chemotherapy (United States) Jun 2003 , 47 (6) p1936-42 ,
ISSN: 0066-4804--Print **Journal Code:** 0315061
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Lamivudine [beta-L-(-)-2',3'-dideoxy-3'-thiacytidine] is a potent inhibitor of hepadnavirus replication and is used both to treat chronic hepatitis B virus (**HBV**) infections and to prevent reinfection of transplanted livers. Unfortunately, lamivudine-resistant **HBV** variants do arise during prolonged therapy, indicating a need for additional antiviral drugs. Replication-competent **HBV** constructs containing the reverse transcriptase domain **L180M**/M204V and M204I (rtL180M/M204V and rtM204I) mutations associated with lamivudine resistance were used to produce stable cell lines that express the resistant virus. These cell lines contain stable integrations of **HBV** sequences and produce both intracellular and extracellular virus. **HBV** produced by these cell lines was shown to have a marked decrease in sensitivity to lamivudine, with 450- and 3,000-fold shifts in the 50% inhibitory concentrations for the rtM204I and rtL180M/M204V viruses, respectively, compared to that for the wild-type virus. Drug assays indicated that the lamivudine-resistant virus exhibited reduced sensitivity to penciclovir [9-(4-hydroxy-3-hydroxymethyl-but-1-yl) guanine] but was still inhibited by the nucleoside analogues CDG (carbocyclic 2'-deoxyguanosine) and abacavir ([1S,4R]-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-me thanol). Screening for antiviral compounds active against the lamivudine-resistant **HBV** can now be done with relative ease.

10/3,AB/14 (Item 1 from file: 73) **Links**
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12734972 **EMBASE No:** 2004334563
A novel pattern (sW195a) in surface gene of HBV DNA due to YSDD (L180M plus

M204S) mutation selected during lamivudine therapy and successful treatment with adefovir dipivoxil [1]

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Journal of Clinical Virology (J. CLIN. VIROL.) (Netherlands) 2004 , 31/1 (76-77)
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Number Of References: 4

10/3,AB/15 (Item 1 from file: 5) **Links**
Fulltext available through: USPTO Full Text Retrieval Options SCIENCEDIRECT
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0016160274 **Biosis No.:** 200600505669
Evolution of multi-drug resistant HBV: Implications on rescue therapy

Author: Yim Hyung Joon; Hussain Munira; Wong Stephen; Liu Ying; Fung Scott K; Lok Anna S
Journal: Gastroenterology 130 (4, Suppl. 2): p A748-A749 APR 2006 2006
Conference/Meeting: Digestive Disease Week Meeting/107th Annual Meeting of the American-Gastroenterological-Association Los Angeles, CA, USA May 19 -24, 2006; 20060519
Sponsor: Amer Gastroenterol Assoc Inst
ISSN: 0016-5085
Document Type: Meeting; Meeting Abstract
Record Type: Abstract
Language: English

Abstract: Background: Multi-drug resistant **HBV** have been reported in patients who received sequential treatment with nucleoside monotherapy. In vitro studies showed that **HBV** constructs with mutations resistant to lamivudine (LAM) and adefovir (ADV) have marked reduction in sensitivity to combination of LAM+ADV, while constructs with mutations resistant to either drug remain sensitive to the other drug. Aims: To determine if mutations conferring resistance to multiple antiviral agents are present on the same **HBV** genome in vivo and to describe the evolution of these mutations. Methods: Sera from 6 **HBV** mutations on direct sequencing were cloned after nested PCR, 18-20 clones from each sample were sequenced. Results: Mutations to both therapies were present on the same genome in 163/195 (84%) clones from 10 samples with dual-resistant mutations to LAM+ADV, LAM+HBIG, or LAM+entecavir (ETV) on direct sequencing, 32 (16%) clones had mutations to one drug. Evolution of mutations was examined in 3 patients. Patient I received LAM+ETV after LAM breakthrough, all 18 clones had **L180M** and M204V/I at month 0 (start of ETV), clonal analysis first detected ETV-resistant mutation (T184L) at month 20, 6 months earlier than direct sequencing. Both treatments were stopped at month 34. (T184L: 20/20 clones); 6,months later, T184L was detected in 12/20 clones while **L180M**

and M204V/I remained detectable in 19/20 clones. Patient 2 was switched to ETV monotherapy after LAM breakthrough, all 20 clones had **L180M**+M204V at month 0. At month 36, ETV-resistant mutation I169T was detected in 15 and S202G in 4 clones. At month 41, S202G was present in 17 clones and I169T in 4 clones, LAM-resistant mutations remained detectable in all 20 clones. Patient 3 developed **HBV** recurrence after transplant despite receiving LAM+HBIG. All 18 clones had M204I and sG145R when **HBV** recurrence was diagnosed. ADV was added and LAM stopped 7 months later. ADV breakthrough occurred after 41 months of ADV when all 18 clones had ADV-resistant N236T. Four months after reintroduction of LAM, all 20 clones had **L180M**+M204V, 12 clones had additional V173L change. However, N236T was replaced by a different ADV-resistant mutation P237H. Conclusions: Our study showed that mutations conferring resistance to multiple antiviral agents are present on the same viral genome, suggesting that combination therapy directed against mutants resistant to each treatment may not be adequate in suppressing dual-resistant **HBV**. Sequential antiviral therapy leads to selection of multi-resistant **HBV**, mutations evolve during continued treatment resulting in mutants with increased replication fitness.

10/3,AB/16 (Item 2 from file: 5) **Links**
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0014764924 **Biosis No.:** 200400132278
Kinetics of viral resistance and serum HBV DNA in patients underlamivudine therapy.

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Journal: Hepatology 38 (4 Suppl. 1): p 719A October 2003 2003
Medium: print
Conference/Meeting: 54th Annual Meeting of the American Association for the Study of Liver Diseases Boston, MA, USA October 24-28, 2003; 20031024
Sponsor: American Association for the Study of Liver Diseases
ISSN: 0270-9139 _(ISSN print)
Document Type: Meeting; Meeting Abstract
Record Type: Abstract
Language: English

Abstract: Aim: to assess the kinetics of emergence of viral resistance and the kinetics of serum **HBV** DNA using a sensitive assay for serum **HBV** DNA quantification, in patients with chronic hepatitis B treated with Lamivudine. Methods: Among 150 **HBV**-infected patient treated with Lamivudine in our clinic, 28 who had a phenotype of viral resistance (initial virological response followed by reappearance of detectable serum **HBV** DNA with a standard hybridization assay VERSANTR **HBV** Assay v1.0 (bDNA) (sensitivity 700.000 **HBV** DNA copies/ml) were tested retrospectively at serial time points from initiation of treatment to viral breakthrough. Genomic sequence analysis was performed using the TRUGENE **HBV** Genotyping Kit (Bayer Healthcare-Diagnostics). Viral load was assessed

with VERSANTR **HBV** Assay v3.0 (bDNA) (Bayer Healthcare-Diagnostics) sensitivity 2,000 **HBV** DNA copies/ml. Results: At treatment initiation, none of the patients demonstrated the presence of viral resistance. At viral breakthrough, 15.7+-7.9 months (range 7 to 36) after treatment initiation, 25 patients demonstrated the presence of **HBV** variants with mutation in the YMDD motif and were studied further. They consisted of 23 men 2 women with a mean age of 45+-12 (23-69) years. Six patients had HIV co-infection and 5 were HBeAg negative. Sequence analysis was successfully carried out in 120 sequential serum samples tested. Genotype A was found in 14 patients (56%) and genotypes B, C, D, and E in 11 patients. Mutation M204V in pol gene was found in 15 patients and was al ways associated with an **L180M** substitution. Mutation M204I was found in the remaining 10 patients, and was not associated with other mutations. In the 23 patients tested sequentially, before the viral breakthrough serum **HBV** DNA became undetectable with VERSANTR **HBV** Assay vl.0 in all patients but remained persistently detectable in 17/23 when using VERSANTR **HBV** Assay v3.0. The sensitivity of the sequencing assay allowed sequence analysis of serum samples with viral load <2000 **HBV** copies/ml. Mean serum **HBV** DNA levels were: 4.6+-1.6 (0.7-6.7)-log10 **HBV** copies/ml and 4.7+-1.6 (0.2-6.6) log10 **HBV** copies/ml at the timepoint when the patients demonstrated no viral resistance and at the timepoint showing the first viral resistance, respectively. The presence of mutation in the YMDD motif was observed as early as 4.1+-2.7 months (range 1 to 12) before viral breakthrough. No correlation was found between the profile of YMDD substitution and the delay for viral breakthrough or the presence of HIV co-infection. Conclusions: In patients under Lamivudine therapy our results show that the detection of viral resistance is possible 1- precociously (1 to 12 months before viral breakthrough) 2- with very low serum **HBV** DNA titers (<2000 **HBV** copies/ml); 3- before serum **HBV** DNA increase. These results suggest that monitoring of viral load with a sensitive quantitative assay together with the use of a sensitive assay for the detection of viral resistance could be clinically relevant (rapid adjustment of antiviral therapy).

10/3,AB/17 (Item 3 from file: 5) **Links**
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0014764918 **Biosis No.:** 200400132272

In vitro susceptibility of wild-type and lamivudine-resistant hepatitis B virus to tenofovir and adefovir.

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Author Address: GH Pitie-Salpetriere, Paris, France**France
Journal: Hepatology 38 (4 Suppl. 1): p 717A October 2003 2003
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Sponsor: American Association for the Study of Liver Diseases
ISSN: 0270-9139 _(ISSN print)

Document Type: Meeting; Meeting Abstract
Record Type: Abstract
Language: English

Abstract: Emergence of lamivudine-resistant hepatitis B virus (**HBV**) is of great concern in human immunodeficiency virus (HIV) and **HBV** coinfected patients; the purpose of this study was to assess the in vitro efficacy of new nucleotide analogues on **HBV** strains isolated from lamivudine-treated patients. HIV-coinfected patients analysed were enrolled in an open label trial of adefovir dipivoxil (ADV) for the treatment of lamivudine-resistant (lam-R) hepatitis B. After purification of **HBV** DNA from patient serum, the whole **HBV** genome was PCR amplified and cloned. Drug sensitivity was measured after transfection of the isolated full genomes into HepG2 cells and measurement of HBe Ag, HBs Ag and viral replication in the culture media under increasing drug concentrations. A wild type (wt) strain isolated from an untreated patient served as control. In our system, lamivudine-associated resistance mutations (rtL180M and rtM204V) increased by more than 16,000 fold the IC50 of lamivudine (from 6nM to over 100mu M) when compared to wt **HBV**. The most common lam-R mutations (**L180M**-M204V) induced a slight decrease of both adefovir and tenofovir activity. Comparison of IC50 of wt and lam-R **HBV** showed a 2.85 (from 0.07 to 0.2mu M) and a 3.3 (from 0.06 to 0.2mu M) fold increase for adefovir and tenofovir, respectively. Three patients who had poor response to ADV treatment (less than 2.5 Log copies/mL **HBV**-DNA drop, 3 months after ADV introduction) were further studied. One of them had the rtL180M and rtM204V mutations while the others carried also a compensatory mutation rtV173L. In vitro, adefovir was less active on strains carrying the V173L mutation associated with **L180M**/M204V compared to the sole double mutant **L180M**/M204V. At the adefovir concentration of 0.1mu M, presence of the V173L mutation reduced the inhibition of HBs Ag production from 50 to 30% ($p<0.01$) and the viral replication from 45 to 32% ($p<0.01$). Conversely, tenofovir had similar potency on both **HBV** mutation profiles with 60% inhibition of HBs Ag production and 45% inhibition of viral replication at 0.1mu M. No data were available on the in vitro efficacy of tenofovir on lam-R **HBV**. Our study supports the high efficacy of ADV and TDF seen in patients after lamivudine breakthrough. The enhanced activity of tenofovir compared to adefovir on virus carrying the mutation pattern rtV173L/**L180M**/M204V, often found in HIV-**HBV** patients with lamivudine resistance, offers an interesting treatment alternative to HIV-**HBV** coinfected patients.

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0014660999 **Biosis No.:** 200400031756

COMPARISON OF VIRAL REPLICATION FITNESS OF WILD-TYPE AND LAMIVUDINE-RESISTANT HBV IN PATIENTS .

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Author Address: Foster City, CA, USA**USA
Journal: Digestive Disease Week Abstracts and Itinerary Planner 2003 p Abstract No. 339 2003 2003

Medium: e-file
Conference/Meeting: Digestive Disease 2003 FL, Orlando, USA May 17-22, 2003; 20030517
Sponsor: American Association for the Study of Liver Diseases
American Gastroenterological Association
American Society for Gastrointestinal Endoscopy
Society for Surgery of the Alimentary Tract
Document Type: Meeting; Meeting Abstract
Record Type: Abstract
Language: English

Abstract: Background: Lamivudine-resistant **HBV** has been described as replication defective based on early clinical observations and in vitro data for single mutations of M204V or M204I in the YMDD motif of **HBV** polymerase. However, recent data suggests that compensatory mutations also emerge and patients with long-term lamivudine resistance undergo disease progression. Aims: To compare levels of serum **HBV** DNA and ALT in patients with lamivudine-resistant and wild-type **HBV**. To determine the prevalence of compensatory mutations in YMDD-mutants and their contributions to replication fitness. Methods: Patients analyzed were enrolled in trials of adefovir dipivoxil for the treatment of wild-type (2 trials, n=695) or lamivudine-resistant **HBV** (3 trials, n=203). Patients were required to have serum **HBV** DNA levels gtoreq5 log10 copies/mL at entry. Serum **HBV** DNA was measured by Roche Amplicor PCR. DNA sequencing was used to identify resistance mutations at baseline. Results: At baseline, patients with lamivudine-resistant **HBV** had similar serum **HBV** DNA (medians 7.7, 8.1, and 8.8 log10 copies/mL) compared to patients with wild-type **HBV** (medians 7.1 and 8.4 log10 copies/mL); median ALT levels were 79-82 IU/L in lamivudine-resistant and 94-98 IU/L in wild-type patients. Compensatory mutations (V173L, **L180M**) that enhance the replication of YMDD-mutant **HBV** in vitro were found in the majority (88%) of lamivudine-resistant patients. At baseline, four major mutational patterns were identified in patients with lamivudine-resistant **HBV** (**L180M**+M204V (58%), V173L+ **L180M**+M204V (17%), M204I (12%), **L180M**+M204I (11%)); serum **HBV** DNA did not vary significantly across the different mutational patterns. Conclusions: Lamivudine-resistant **HBV** is capable of replicating to levels comparable to wild-type **HBV** and causing abnormally high ALT. Exclusion of patients with lower serum **HBV** DNA levels from eligibility preclude an assessment across a greater spectrum of viral load. The four patterns of lamivudine resistance mutations all had similar levels of serum **HBV** DNA..

10/3,AB/19 (Item 5 from file: 5) **Links**
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0014633946 **Biosis No.:** 200400004703

Emerging amino acid substitutions in lamivudine-resistant HBV that result in reduced susceptibility to entecavir.

Author: Colonno R J (Reprint); Weinheimer S (Reprint); Rose R (Reprint); Levine S (Reprint); Discotto L (Reprint); Plym M (Reprint); Yu C (Reprint); Tenney D (Reprint); Angus P; Sievert W; Bartholomeusz A; Ayres A; Warner N; Thompson G; Sozzi V; Edwards

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Journal: Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy 43 p 497 2003 2003
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Conference/Meeting: 43rd Annual Interscience Conference on Antimicrobial Agents and Chemotherapy Chicago, IL, USA September 14-17, 2003; 20030914
Sponsor: American Society for Microbiology
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Record Type: Abstract
Language: English

Abstract: Background: Entecavir (ETV) demonstrated potent antiviral activity in lamivudine resistant (LVDR) patients with chronic hepatitis B virus (**HBV**) infection. Among the >500 ETV-treated patients in phase II trials, 2 heavily pretreated patients exhibited a rebound in viral load and were further evaluated. Methods: Genotypic analysis involved sequencing the reverse transcriptase (RT) gene from serum **HBV** DNA samples. Antiviral assays in HepG2 cells used transient transfection or baculovirus mediated expression of recombinant **HBV**. Results: "Patient A" received 0.5 mg ETV for 52 wk with apprx2 log **HBV** DNA reduction, treatment then included 0.5 mg ETV+LVD before displaying a rebound at wk 100. LVDR substitutions **L180M**, M204V and V173V/L were present at study entry, with substitutions I169T (B Domain) and M250V (E Domain) selected on treatment. Reduced ETV susceptibility required M250V in addition to LVDR mutations. Liver transplant "Patient B" failed famciclovir, ganciclovir, foscarnet and LVD therapy and had RT changes S78S/T, **L180M**, V173V/L, T184T/S and M204V at study entry. Viral rebound occurred after 80 wk of ETV (1.0 mg) therapy with the additional substitutions A38E, T184G (B domain) and S202I (C domain) selected. Phenotypic assays showed reduced ETV susceptibility when both the T184G and S202I changes were combined with the LVDR mutations. Recombinants encoding subsets of these changes were **HBV** replication impaired and retained ETV susceptibility. Conclusion: Additional RT mutations that can lead to reduced ETV susceptibility appear to emerge infrequently on a LVDR backbone following prolonged ETV treatment in heavily pretreated patients.